Evaluation and refinement of a field-portable drinking water toxicity sensor utilizing electric cell–substrate impedance sensing and a fluidic biochip

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ABSTRACT: The US Army's need for a reliable and field-portable drinking water toxicity sensor was the catalyst for the development and evaluation of an electric cell–substrate impedance sensing (ECIS) device. Water testing technologies currently available to soldiers in the field are analyte-specific and have limited capabilities to detect broad-based water toxicity. The ECIS sensor described here uses rainbow trout gill epithelial cells seeded on fluidic biochips to measure changes in impedance for the detection of possible chemical contamination of drinking water supplies. Chemicals selected for testing were chosen as representatives of a broad spectrum of toxic industrial compounds. Results of a US Environmental Protection Agency (USEPA)-sponsored evaluation of the field portable device were similar to previously published US Army testing results of a laboratory-based version of the same technology. Twelve of the 18 chemicals tested following USEPA Technology Testing and Evaluation Program procedures were detected by the ECIS sensor within 1 h at USEPA-derived human lethal concentrations. To simplify field-testing methods further, elimination of a procedural step that acclimated cells to serum-free media streamlined the test process with only a slight loss of chemical sensitivity. For field use, the ECIS sensor will be used in conjunction with an enzyme-based sensor that is responsive to carbamate and organophosphorus pesticides. Copyright © 2014 John Wiley & Sons, Ltd.

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Keywords: impedance; RTgill-W1; water toxicity; ECIS; toxicity sensor; *in vitro* toxicology; rainbow trout gill epithelial cells; fluidic biochip

Introduction

The US Army has been pursuing the development of simple and reliable field methods for rapid detection of chemical toxicity in drinking water. Water testing technologies historically available to soldiers in the field are analyte-specific (Kelly *et al*., 2008; Pancrazio *et al*., 1999; States *et al*., 2003) and have limited detection capabilities. Recently developed toxicity sensors with broad-based detection potential (Brennan *et al*., 2012; Curtis *et al*., 2009a, 2009b, 2013; Iuga *et al*., 2009; van der Schalie *et al*., 2006) have been considered and evaluated by the US Army to address this technology gap. A recently described water toxicity testing method (Brennan *et al*., 2012) demonstrated that a cell-based toxicity sensor using electric cell–substrate impedance sensing (ECIS) and fluidic biochips containing rainbow trout gill epithelial cells (RTgill-W1) had the potential to be developed into a field-portable broad-based biosensor. Important characteristics of the biosensor for field implementation include rapid response time (less than 1 h), sensitivity to toxic chemicals at concentrations relevant to human health, ease of use and maintenance-free long-term storage capability of the biological components.

The ECIS field sensor described here operates on the same principle as the laboratory-based version described previously (Brennan *et al*., 2012), but now utilizes a hand-held reader. Fluidic biochips with confluent monolayers of the RTgill-W1 cells

growing on sensing electrodes in the chip interface with the reader. The ECIS reader (15 kHz at 1 mV) measures changes in the electrical impedance of the cell layers when toxic chemicals are introduced into the biochip. Impedance measurements correlate with changes in cellular morphology, membrane permeability and cell attachment factors (Giaever and Keese, 1984, 1991, 1993; Keese *et al*., 1998; Narakathu *et al*., 2010; Xiao and Luong, 2003, 2005), all of which can be affected by toxic insults. Changes in impedance occur when the integrity of the cell monolayer on the biochip is compromised. The RTgill-W1 cells, which make up the biological component of sensor, were chosen over other mammalian cell lines based on previous testing done with laboratory-based ECIS sensors, where RTgill-W1 sensitivity to toxic chemicals during ECIS testing was comparable to a bovine cell line (Brennan *et al*., 2012; Curtis *et al*., 2009b). As the RTgill-W1 cells are derived from

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14. ABSTRACT

The US Army???s need for a reliable and field-portable drinking water toxicity sensor was the catalyst for the development and evaluation of an electric cell-substrate impedance sensing (ECIS) device. Water testing technologies currently available to soldiers in the field are analyte-specific and have limited capabilities to detect broad-based water toxicity. The ECIS sensor described here uses rainbow trout gill (RTgill-W1) cells seeded on fluidic biochips to measure changes in impedance for the detection of possible chemical contamination of drinking water supplies. Chemicals selected for testing were chosen as representatives of a broad spectrum of toxic industrial compounds. Results of a US Environmental Protection Agency (USEPA)-sponsored evaluation of the field portable device were similar to previously published Army testing results of a laboratory-based version of the same technology. Twelve out of the eighteen chemicals tested following USEPA Technology Testing and Evaluation Program (TTEP) procedures were detected by the ECIS sensor within 1 h at USEPA-derived human lethal concentrations. To further simplify field-testing methods, elimination of a procedural step that acclimated cells to serum-free media streamlined the test process with only a slight loss of chemical sensitivity. For field use, the ECIS sensor will be used in conjunction with an enzyme-based sensor that is responsive to carbamate and organophosphorus pesticides

15. SUBJECT TERMS
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poikilothermic animals (Bols *et al.*, 1994), they do not require a 37 °C incubator for survival, nor do they need the high levels of carbon dioxide (5–10%) or frequent media replenishments required by mammalian cells. In addition, fluidic chips containing monolayers of RTgill-W1 cells could be stored at refrigerated temperatures for up to 78 weeks with no media replenishments and were still viable sensors for water toxicity testing (Brennan *et al.*, 2012). All of these characteristics made the RTgill-W1 cells ideal candidates for the biological components of a field-portable water toxicity biosensor.

For the ECIS toxicity sensor to proceed to advanced development and fielding, it had to first meet several US Army user requirements, including independent laboratory testing using USEPA Technology Testing and Evaluation Program (TTEP) procedures (US EPA, 2008). This paper presents the results of toxicity testing with the ECIS sensor done by Battelle (Columbus, OH, USA) using TTEP procedures, as administered by the USEPA National Homeland Security Research Center. Also presented is an evaluation of whether removing a pre-test procedure to simplify the test would decrease ECIS sensor response to toxicants.

Materials and methods

Fluidic biochips seeded with RTgill-W1 cells

Fluidic biochips were developed by and are commercially available from Biosentinel, Inc. (Austin, TX, USA). The biochips include an upper polycarbonate layer with two separate fluid channels, and a lower electronic layer that contains the gold impedance sensing electrodes. There are four electrode pads per channel with 10 working electrodes on each pad that are 250 μm in diameter. The cells grow on the bottom surface of the two channels. During testing, one channel is dedicated to the control sample and the other is dedicated to the test sample. The impedance levels of the four electrode pads in each of the channels are monitored every 60 s, and differences in impedance levels between channels are determined in real-time (during a 60 min test) using a custom-developed curve discrimination program imbedded within the ECIS software. The statistical program is described in detail elsewhere (Curtis *et al.*, 2009a).

RTgill-W1 cells were obtained from the American Type Tissue Culture Collection (ATCC; Manassas, VA, USA). The cells were cultured in 75 cm^2 polystyrene flasks in complete Leibovitz-15 (L-15) growth media containing 10% fetal bovine serum, 100 U ml^{-1} penicillin and 100 U ml^{-1} streptomycin (all purchased from Lonza, Walkersville, MD, USA) and 2 mM Gluta-MAX-1 supplement (Life Technologies, Inc., Grand Island, NY, USA) in a 20 °C incubator with ambient carbon dioxide. The cells were used at passages 6–16 for seeding the fluidic biochips. Procedures used in this study for seeding fluidic biochips with RTgill-W1 cells are described in detail previously (Brennan *et al.*, 2012). Before being seeded with cells, the channels of the fluidic biochips were coated with an adhesive substrate consisting of 0.01% fibronectin (Calbiochem, Gibbstown, NJ, USA) solution in L-15 media for 1 h to facilitate cell attachment to the biochip electrode and the subsequent formation of a continuous epithelial monolayer. After 1 h incubation at room temperature, the fibronectin solution was aspirated off using a sterile pipet tip under vacuum, and each biochip channel was seeded with 2.5 ml of trypsinized RTgill-W1 cells at a concentration of 2.5×10^5 cells ml^{-1} in complete L-15 growth media using

a sterile syringe. Sterile Pharmed® BPT tubing (Saint-Gobain Performance Plastics, Bridgewater, NJ, USA) was used to form closed loops on the barbed inlets of the biochip channels. Previous unpublished studies have shown that bare electrodes containing culture media and no cells had impedance values of 300–400 ohms. Once the RTgill-W1 cells were introduced into the fluidic biochip and a monolayer was formed, impedance values ranged from approximately 1200 to 2000 ohms 1 week after seeding. Seeded biochips were incubated at 20 °C for 7 days. The growth media in the fluidic channels was replenished on days 4 and 7 before storing the biochips at 6 °C on day 7.

Field-portable electric cell–substrate impedance sensing reader

The field-portable ECIS readers used in the studies reported here were developed by Biosentinel, Inc., and Nanohmics, Inc. (Austin, TX, USA) in collaboration with the US Army Center for Environmental Health Research (USACEHR; Fort Detrick, MD, USA). Reader dimensions are 14 cm × 22.9 cm × 8.9 cm with a weight of 1.4 kg. The reader can function on battery power or with standard 110 V power and has a USB data transfer port. The fluidic biochips are inserted into the reader to engage an edge-card connector for electrical interfacing to facilitate impedance measurements, which are recorded and displayed on a touch screen once per minute. Test results for water samples are displayed as either "not contaminated" after a 1 h test period, or as "contaminated" as soon as identified by the software (10 min to 1 h after the start of the test).

Test chemicals

The 18 chemicals tested previously by USACEHR (Brennan *et al.*, 2012) using a laboratory-based version of the ECIS reader were selected for the USEPA TTEP evaluation studies at Battelle using the field-portable ECIS reader. For the TTEP evaluation studies, the chemicals were tested at three concentration levels; the Military Exposure Guideline (MEG), the Army's Human Lethal Concentration (AHLC) and the USEPA's Human Lethal Concentration (EPAHLC). The MEG concentration is considered to be a threshold above which adverse health effects may occur if a soldier were to consume 15 liters of water per day for 7–14 days (US Army Public Health Command, 2013). The AHLC is a toxicological lethal concentration based on consuming 15 liters of water per day for a 70 kg person (TERA, 2006). The EPAHLC is determined from an estimated toxicological lethal dose (based on a rodent LD$_{50}$) at a concentration that would be consumed in 250 ml of water. The 18 chemicals, levels of detection and the MEGs, AHLCs and EPAHLCs are listed in Table 1. Acrylonitrile, aldicarb, arsenic (sodium arsenite), azide (sodium azide), copper (copper sulfate), fenamiphos, fluoroacetate (sodium), methamidophos, methyl parathion, nicotine, paraquat (dichloride), pentachlorophenate (sodium; PCP), phenol, thallium and toluene were all purchased from Chem Service (West Chester, PA, USA). Ammonia (ammonium chloride), cyanide (sodium) and mercury (chloride) were all purchased from Sigma-Aldrich (St. Louis, MO, USA).

A subgroup of nine chemicals used in the USEPA TTEP evaluation studies – ammonia, arsenic, azide, copper, cyanide, mercury, methyl parathion, PCP and toluene – were tested at USACEHR as part of an effort to simplify the ECIS test by removing a pre-exposure procedure, as described below.

Table 1. Summary of ECIS sensor responses to chemicals

Contaminant	Number of ECIS positive responses[a] (concentration tested, mg l^{-1})					Brennan, et al., detection limit (mg l^{-1})[f]
	EPAHLC	0.1× EPAHLC[b]	0.01× EPAHLC[c]	AHLC	MEG	
Acrylonitrile[g]	2 (7000)	NA	NA	0 (4.2)	2 (0.47)	(405)
Aldicarb[g]	1 (168)	0	NA	0 (0.17)	0 (0.0047)	(663)
Ammonia (ammonium chloride)[h]	4 (88 600)	NA	NA	4 (924)	0 (30)	(100)
Arsenic (sodium arsenite)[g]	4 (3000)	4 (300)	4(30)	0 (4.5)	1 (0.02)	(4.5)
Azide (sodium azide)[h]	4 (8000)	NA	NA	4 (47)	2 (0.12)	(12)
Copper (sulfate)[h]	4 (35 000)	NA	NA	1 (103)	1 (0.047)	(1)
Cyanide (sodium)[h]	4 (250)	NA	NA	4 (14)	0 (2)	(14)
Fenamiphos	NA	4 (70)	1 (7)	1 (0.56)	1 (0.0042)	(5.6)
Fluoroacetate (sodium)[h]	0 (200)	NA	NA	0 (5.1)	0 (0.00072)	(510)
Mercury (chloride)[h]	4 (280)	NA	NA	4 (24.7)	0 (0.01)	(0.247)
Methamidophos[g]	0 (3000)	NA	NA	0 (1.4)	0 (0.00023)	(6605)
Methyl parathion[g]	NS	NS	NS	4 (10)[d]	1 (0.14)	(21.4)
Nicotine[g]	0 (280)	NA	NA	1 (16.8)	0 (0.13)	(1000)
Paraquat (dichloride)[g]	3 (800)	NA	NA	3 (4.6)	0 (0.034)	(460)
Pentachlorophenate (sodium) (PCP)[h]	NS	4 (1000)	NA	4 (71.9)	4 (0.14)[e]	(2.5)
Phenol[h]	4 (39 000)	4 (3900)	0 (390)	0 (91.5)	1 (2.8)	(368)
Thallium (sulfate)[h]	NS	4 (280)	NA	1 (13.5)	0 (0.0033)	(27)
Toluene[h]	NS	NA	NA	4 (427)	0 (9.3)	(100)

AHLC, US Army Human Lethal Concentration; EHLC, US Environmental Protection Agency Human Lethal Concentration; ECIS, electric cell–substrate impedance sensing; MEG, Military Exposure Guideline; NA, not analysed; NS, not soluble.
[a]Of four tests on replicate samples.
[b]EHLC diluted 10×.
[c]EHLC diluted 100×.
[d]Actual AHLC is 33.6 mg L^{-1}, but not soluble at that concentration.
[e]Results of tests using a 10× dilution of the MEG concentration were negative.
[f]Detection limit determined by lowest concentration were 16 of 16 positive replicates were detected if at or below the AHLC or three of three positive replicates if detected above the AHLC.
[g]Chem Service, West Chester, PA, USA.
[h]Sigma-Aldrich, St. Louis, MO, USA.

Test samples were prepared by adding neat chemicals to ASTM Type II water (ASTM, 2007), hereafter referred to as deionized (DI) water, to produce desired test concentrations. A sample of each stock solution was confirmed through analytical measurement for both TTEP testing and for USACEHR studies. On the day of testing, stock solutions were diluted using DI water to obtain the concentrations noted in Table 1.

Interferences

Interferences are defined as chemicals or water quality conditions commonly found in some water sources that are unlikely to cause human health effects, but could interfere with sensor detection of toxicants. Table 2 lists six interferences and the concentration levels selected for evaluation under TTEP. Potential interferences tested were chlorine and chloramine (commonly used for drinking water disinfection), geosmin and 2-methyl-isoborneol (MIB) (byproducts of cyanobacteria blooms) and humic/fulvic acids (by-products of plant decomposition). Hard water, which is high in calcium and magnesium and associated anions, was included because of the potential sensitivity of some biological systems. Chlorine, geosmin and MIB were purchased from Sigma-Aldrich, and humic/fulvic acids

Table 2. Electric cell–substrate impedance sensing interferences response summary

Interference	Concentration tested (mg L^{-1})	Positive responses[a]
Chlorine	10	0
Chloramine	10	4
	5	1
Geosmin	0.0001	0
Methyl-isoborneol	0.0001	0
Humic acid and fulvic acid	2.5/2.5[b]	1
Hardness (as CaCO$_3$)	250	1

[a]Of four tests on replicate samples.
[b]Concentrations are 1 : 1 weight by weight.

were purchased from International Humic Substances Society (St. Paul, MN, USA). Chloramine was prepared from the reaction of chlorine and ammonia using a method described by Gordon et al. (1992). Water with a hardness of 250 mg l^{-1} CaCO$_3$ was prepared and measured according to instructions in ASTM Standard E 729–96 (2007). USACEHR verified their chlorine and

chloramine concentrations using a Wallace and Tiernan® Series A790 Amperometric Titrator (Siemens Water Technologies Corp. Alpharetta, GA, USA). TTEP verified their chlorine and chloramine concentrations using a Hach DR-890 (Hach Company, Loveland, CO, USA). Geosmin, MIB and humic/fulvic acids were tested at nominal concentrations.

Positive and negative controls

Quality control samples were tested intermittently throughout the TTEP evaluation to determine a false positive and a false negative rate for the ECIS field-portable reader. DI water in L-15ex media was used for negative control samples. A A 4.9 g l^{-1} solution of sodium chloride that was osmotically equivalent to the L-15ex media was used for positive control samples. The reasoning behind using this osmotically equivalent solution was to demonstrate that the response was cellular and not due to a physical shift in impedance.

Testing procedures

The procedure for ECIS chemical testing using fluidic biochips seeded with RTgill-W1 cells in a laboratory-based reader has been described previously (Brennan *et al.*, 2012); modifications of those procedures are described here. For these studies, a field-portable reader was used instead of a laboratory-based benchtop reader. A Nordson Micromedics Fibrijet® Applicator Assembly (St. Paul, MN, USA) 10 ml syringe holder was used to hold and facilitate uniform and simultaneous injection of control and test samples into the fluidic biochips. Fluidic biochips were removed from 6 °C storage, and testing was performed at ambient room temperature. The fluidic biochips have two channels for testing samples; one channel is used for control samples and the other for test samples. Pre-exposure, control and test solutions were prepared by adding a vial of pre-metered L-15ex powder (US Biological, Swampscott, MA, USA), rather than the concentrated 2 × liquid media used previously (Brennan *et al.*, 2012), to either DI water for pre-exposure and control samples, or to the desired concentration of the test chemical. L-15ex media (Schirmer *et al.*, 1997) has been used for toxicity testing of metals and polycyclic aromatic hydrocarbons with RTgill-W1 cells (Schirmer *et al.*, 1998, 2001; Dayeh *et al.*, 2005), and is a modified version of the complete L-15 growth media used for the culture and feeding of RTgill-W1 cells. The L-15ex media contains the same concentrations of salts, galactose and pyruvate as complete L-15 growth media, but no vitamins, amino acids, serum or antibiotics. Phenol red was also present in the L-15ex media and this facilitated visual comparisons between the color of the control and test samples, as shifts in pH that were greater than 0.20 pH units caused changes in media color. If a color shift in the test media occurred, then those samples were adjusted with 20% HCl or 1.0 N NaOH until the color of the test sample was empirically similar to the control sample to ensure that ECIS responses were due to chemical toxicity and not pH shifts.

TTEP testing utilized a pre-exposure period during which the cell monolayers being maintained in L-15 growth media were injected with L-15ex media and given a 30 min period to acclimate to the new media before the introduction of test chemicals in L-15ex media. Both channels of the fluidic biochip were injected with 10 ml of ambient temperature L-15ex control media over a 20–30 s period using 10 ml syringes in the syringe

holder. The biochip was then inserted into the ECIS reader and impedance data were collected for 30 min. At the end of this pre-exposure period, one channel of the biochip was injected with 10 ml of control L-15ex media and the other channel was injected with 10 ml of L-15ex media dissolved in a test solution containing the test chemical or interference. Simultaneous injections were accomplished using the syringe holder. Impedance data were collected every minute for another 1 h. A chemical or interference was considered as detected if all four biochips tested were positive for contamination at concentrations less than or equal to the EPAHLC, but greater than the MEG. The EPAHLC was not tested when this concentration exceeded the water solubility of the chemical, as was the case for fluoroacetate, methyl parathion, PCP, thallium and toluene (see Table 1). For all tests, the biochips could only be used once and were discarded at the end of the test.

Testing at USACEHR was conducted to determine if the ECIS method could be simplified by eliminating the 30 min pre-exposure acclimation period. The biochip was directly inserted into the reader while the cell monolayers were still in an environment of complete L-15 growth media and subsequently injected with either control or test solution in L-15ex media. Impedance data were collected for 2 min before injecting control and test media as described in the TTEP protocol. Impedance test data were then collected every min for 1 h. For both the TTEP and the no pre-exposure testing protocols, the reader provided a visual display of "contaminated" or "no contamination detected" for the results of the test samples.

Data acquisition and statistical analysis method

Toxicity of a water sample is indicated by the ECIS reader when the impedance response in the control channel differs significantly from the test channel ($P < 0.001$), as determined by the curve discrimination software in the reader. ECIS sensor software uses a model that takes into account the control sample being analyzed side-by-side with the test sample, as well as incorporating the impedance data files collected from the multiple negative control samples analyzed previously. The method, developed by Dr. Steve Schwager of Cornell University using MATLAB (The Mathematics, Inc., Natick, MA), is described in more detail elsewhere (Curtis *et al.*, 2009a, 2009b).

Results and discussion

USEPA Technology Testing and Evaluation Program evaluation

Table 1 shows the toxicity response results from the USEPA TTEP evaluation testing. The ECIS sensor detected 12 of the 18 toxicants tested within 1 h after the toxicant had been introduced into the biochip. The chemicals and lowest concentrations detected (mg l^{-1}) were ammonia (924), arsenic (30), azide (47), copper (35,000), cyanide (14), fenamiphos (70), mercury (24.7), methyl parathion (10), PCP (0.14), phenol (3900), thallium (280) and toluene (427). Seven of the 18 toxicants were detected after 1 h of exposure at concentrations less than or equal to the AHLC, but greater than or equal to the MEG. These chemicals were ammonia, azide, cyanide, mercury, methyl parathion, PCP and toluene. PCP was detected at the MEG, but was not detected at a concentration an order of magnitude below the MEG.

Overall TTEP results were consistent with those of previously reported results for the laboratory-based ECIS studies performed at USACEHR (Brennan *et al.*, 2012), where a toxicant was considered to be detected when there were 16 of 16 positive fluidic biochip responses from replicate test samples where concentrations were less than or equal to the AHLC, but greater than or equal to the MEG. In the USACEHR tests, nine of the 18 toxicants were detected within 1 h after the toxicant had been introduced into the biochip. The chemicals and concentrations detected (mg l^{-1}) were ammonia (281), arsenic (4.5), azide (12), copper (1), cyanide (14), mercury (0.247), methyl parathion (25.6), PCP (2.5) and toluene (100). When comparing levels of ECIS detection at the AHLC from the different laboratories, arsenic and copper were detected by USACHER but not detected by TTEP. One of the objectives of the USACEHR ECIS sensor testing was to determine the lower level of detection for the sensor, which is why the levels for ammonia, azide, mercury and toluene are reported at concentrations below the AHLC. Cyanide was detected at the same concentration (AHLC) for both laboratories (14 mg l^{-1}). It was found that the solubility of methyl parathion was close to the AHLC of 33.6 mg l^{-1}, and, therefore, it was difficult to test at this concentration. TTEP was able to detect methyl parathion at a concentration of 10 mg l^{-1}, and USACHER was able to detect methyl parathion at a concentration of 25.6 mg l^{-1}. The AHLC of 4.5 mg l^{-1} for arsenic was detected by ECIS in 16 of 16 tests performed by USACEHR, but arsenic was detected at a higher concentration (30 mg l^{-1}) in tests performed by TTEP. Differences noted in arsenic may likely be related to a slightly more variable control model data set for the field-portable ECIS device when compared to the laboratory-based ECIS device (see Fig. 3).

There was greater disparity between TTEP and USACEHR test results for copper. Copper was detected at 1 mg l^{-1} using the laboratory-based instrument at USACEHR, but TTEP testing at 103 mg l^{-1} yielded one positive sample of four. The only other copper concentration tested at TTEP was the EPAHLC (30 000 mg l^{-1}), which was positive (four of four). Interestingly, copper at the AHLC was the only compound tested that yielded an increase in impedance when compared to the control channel during the first hour of exposure; subsequently impedance decreased over the next several hours relative to the control channel. Follow-on testing at USACEHR using the field-portable instrument (with and without a pre-exposure period) resulted in a copper response at 10 mg l^{-1} (see supplementary data).

The interferences (chlorine, chloramine, geosmin, MIB, humic/fulvic acid and hard water) were tested at TTEP. The ECIS sensor did not respond to chlorine, geosmin or MIB at levels that could typically be found in drinking water (Table 2). Chloramine at 10 mg l^{-1} resulted in four of four positive responses. When the concentration of chloramine was reduced to 5 mg l^{-1}, only one of four responses was positive (as was also the case with humic/fulvic acids and hard water). These results suggest that the field-portable reader could be used to test field drinking water chlorinated up to 10 mg l^{-1} and chloraminated up to 5 mg l^{-1}. The US Environmental Protection Agency maximum residual disinfectant level for chlorine and chloramine is set at 4 mg l^{-1} under the Safe Drinking Water Act and thus would not be a detectable interferent in compliant municipal water supplies.

All 35 of the positive control samples tested were positive. A total of 51 negative control samples were tested during the TTEP evaluation. Three negative control samples were positive, which resulted in a false positive rate of 6%. Post-analysis evaluation revealed that two of the false positive samples had outlier impedance readings on one or more of the electrode pads. Visual inspection of the fluidic biochips revealed that one chip had areas of gold flaking, indicating a chip manufacturing problem. Another chip had some anomalous impedance spikes, which may be indicative of a poor electrode connection or a large air bubble traveling over the electrode. Both conditions have been identified as outlier impedance readings and upgrades to the reader software now identify similar outlier data and that the water sample needs to be retested. Furthermore, redesigned fluidic biochips and an improved reader are being developed in collaboration with Biosentinel, Inc. to improve the overall reliability and reproducibility of test results.

Results of testing without a pre-exposure period

The elimination of the pre-exposure period allows the user to perform the initial setup and test sample injection at the initiation of the test, with no further user input required to complete the test. This simplification reduces the test length by 30 min and allows the user to perform other duties without the need to return to the reader 30 min after pre-exposure injections. Figure 1 provides a graphic representation of the results of parallel testing performed with and without a 30 min pre-exposure period for the nine chemicals tested at USACEHR using the ECIS field-portable sensor. The figure depicts the maximum difference in impedance response between the normalized mean of the treatment and control channels for each fluidic biochip. Significant ($P < 0.001$) fluidic biochip chemical responses are depicted with an asterisk.

Using the standard pre-exposure method, eight of the nine chemicals tested were detected at or below the AHLC (mg l^{-1}); ammonia (100), arsenic (4.5), azide (12), copper (10), mercury (2.47), methyl parathion (21.4), PCP (2.5) and toluene (236). Elimination of the pre-exposure period resulted in detection of seven of the nine chemicals tested. There was a reduction in detection capability to azide and ammonia; yielding only two of four positive detects for each chemical. As described below, increased variability in the impedance responses of the no pre-exposure control model fluidic biochips were most likely caused by the reduction in detection to these two chemicals. The elimination of the pre-exposure did, however, result in the detection of cyanide at the AHLC of 14 mg l^{-1}. There was a noticeable difference in the magnitude of response; the elimination of the pre-exposure period resulted in a larger response differential between the control and treatment channels.

Figure 2 illustrates the responses of fluidic biochips to an exposure of 2.47 mg l^{-1} of mercury both with and without a 30 min pre-exposure period. As is typical for no pre-exposure tests, there is a large spike in impedance at the initial injection of the control and mercury samples. This is most likely due to the change in the media in the channels where cells go from complete L-15 media to L-15ex. This impedance response was verified to be biological and not ionic (physical) by repeating the injection on chips that contain no cells. Only a small shift in impedance was detected by the fluidic biochip in the absence of cells.
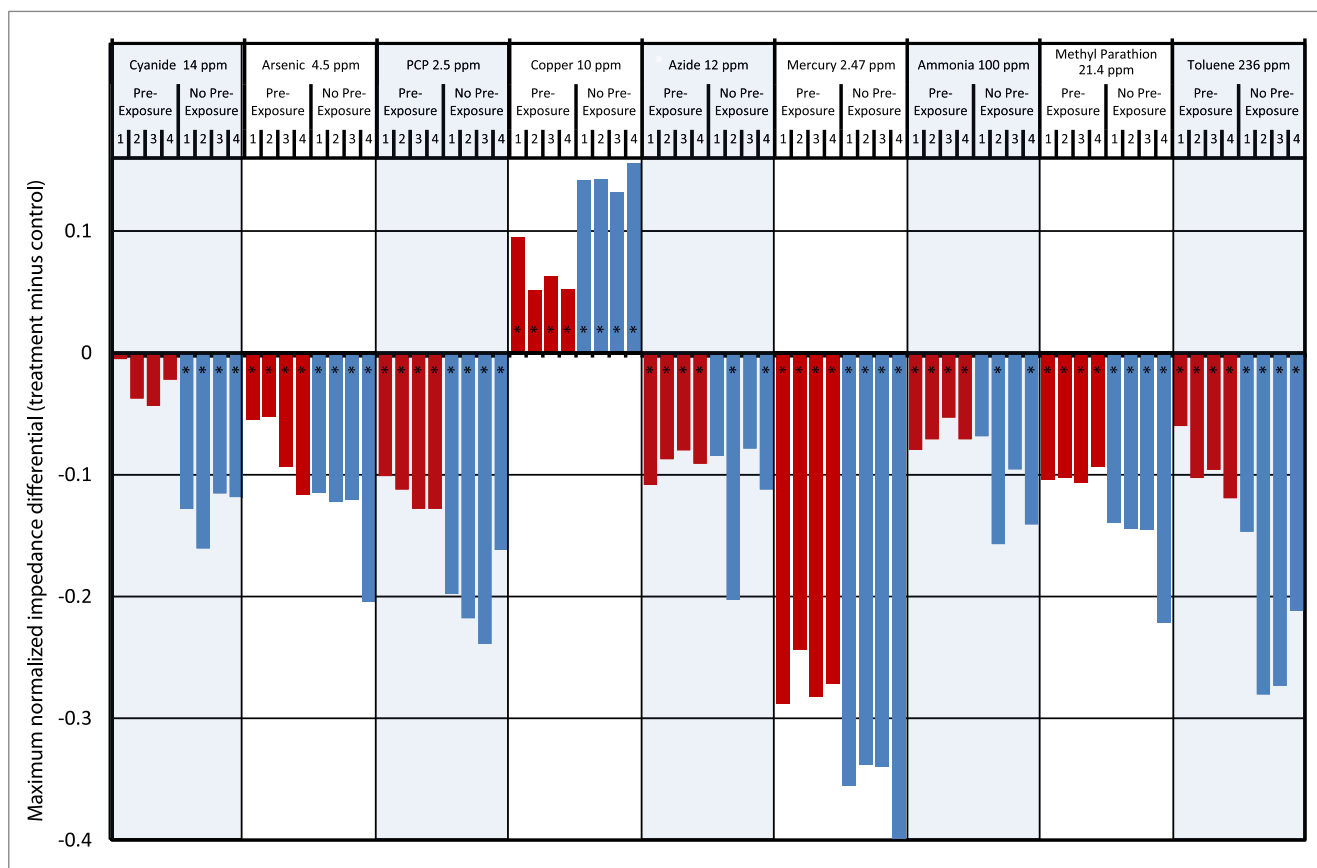
Figure 1. Parallel testing of the pre-exposure and no pre-exposure methods. The maximum difference (either or positive or negative) between the normalized mean of the control and treatment channels of each fluidic biochip is shown. Significant ($P < 0.001$) responses for each fluidic biochip are shown by an asterisk.

Although the magnitude of response was generally greater with the no pre-exposure tests, the variability from biochip to biochip was also greater (Fig. 3). The top graphic displays individual control fluidic biochip data generated using the no pre-exposure method. The lower graphic depicts the same exposure scenario using a 30 min pre-exposure period. The pre-exposure method demonstrates a lower level of variability between fluidic biochips. This reduced variability most likely resulted in the slightly improved detection capability of the pre-exposure method over the no pre-exposure method.
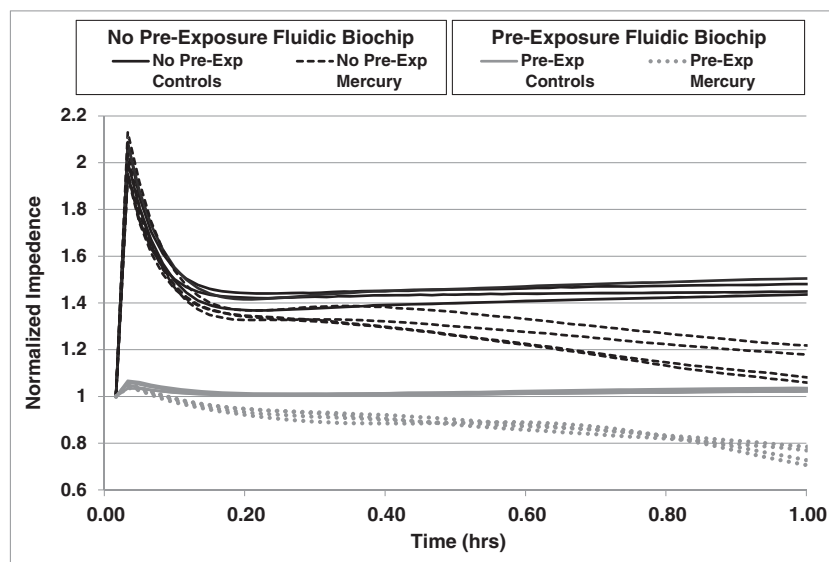
Figure 2. A comparison of fluidic biochips exposed to mercury (2.47 mg l^{-1}) tested with and without the 30 min pre-exposure period.
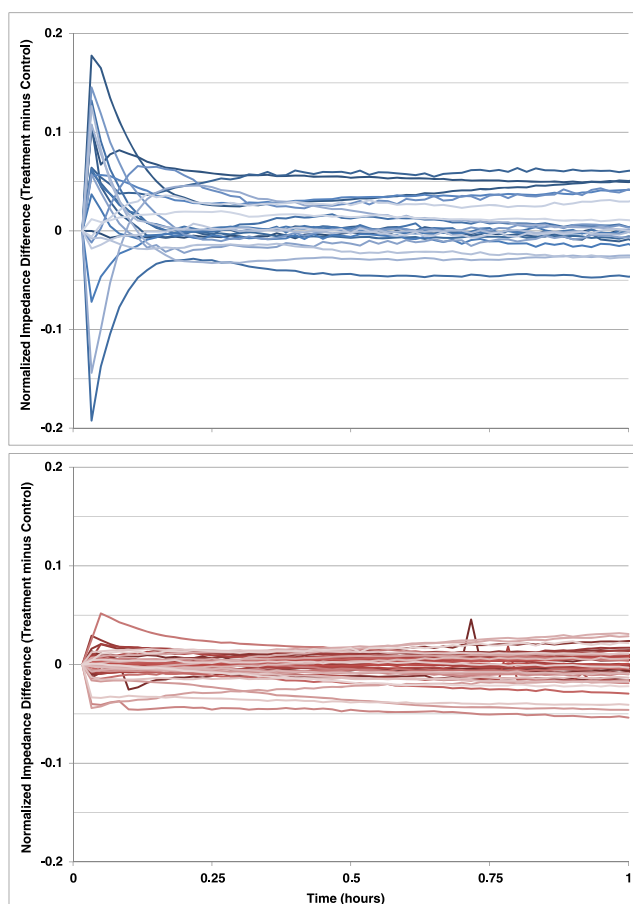
Figure 3. Top (no pre-exposure): Differences in impedance between control and treatment channels in replicate fluidic biochips tested as control blanks without the use of a pre-exposure period. Bottom (pre-exposure): Differences in impedance between control and treatment channels in replicate fluidic biochips tested as control blanks with the use of a pre-exposure period.

Additional considerations

Viability of consumables is an important consideration for US Army applications; while consumables may be refrigerated, they should not require freezing, and they should remain usable for at least 9 months. ECIS testing presented here utilized fluidic biochips that had been in cold storage (6 °C) for several weeks to several months; the biochips have been found to be viable for testing after 78 weeks in cold storage (Brennan *et al.*, 2012). For field use, biochips may be stored and transported in Golden Hour® containers (Minnesota Thermal Science, Plymouth, MN, USA) that are used by the US Army in the field for transport of temperature-sensitive materials, such as blood.

While the USEPA TTEP evaluation demonstrated the capability of the ECIS toxicity sensor to detect rapidly a broad range of chemical toxicants, additional sensitivity to carbamate and organophosphorus pesticides would be desirable. As a result, current plans are to use the ECIS sensor in conjunction with an additional enzyme-based sensor under development by ANP Technologies, Inc.

Eliminating the 30 min pre-exposure period will decrease test length from 90 min to 60 min, reduce the quantity of test materials needed, and limit user involvement for the setup and initiation of the test. Additional improvements are being made

to both the ECIS and enzyme-based toxicity sensors. Before fielding, both sensors will undergo additional TTEP testing at Battelle, as well as environmental testing by the US Army, and a user evaluation to assess the utility of the ECIS sensor under actual field conditions. It is hoped that eventually the ECIS sensor technology will be utilized for testing of both civilian and military drinking water supplies.

Disclaimers

The views, opinions and/or findings contained in this report are those of the authors and should not be construed as official Department of the Army position, policy, or decision, unless so designated by other official documentation. Citations of commercial organizations or trade names in this report do not constitute an official Department of the Army endorsement or approval of the products or services of these organizations.

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